BioHybrid Technologies, Inc.

Treating Disease Using Implanted Living Cells in Microspheres

In the 1990s, treating diseases relied primarily on injections or the oral administration of pharmaceuticals. Certain critical illnesses were treated with organ transplants. In 1993, BioHybrid Technologies, Inc. and its joint venture partner, Synergy Research Corp., submitted a proposal to the Advanced Technology Program (ATP) to develop a form of "cellular medicine," in which they would insert healthy living cells from animal tissue into permeable microspheres (small capsules) and then inject these into patients. They would use this technology to treat diabetes and other potential disease targets, including hemophilia, Parkinson's disease, Alzheimer's disease, liver failure, AIDS, and cancer. Significant technical risks made it difficult to obtain private-sector support. For example, the body's natural immune system might reject the microspheres in spite of the protective coating, or the coatings might not be permeable enough to allow nutrients to move freely and keep the cells alive.

ATP awarded cost-shared funds for a project beginning in 1994. The joint venture successfully developed permeable microspheres and their coatings. This work led to six patent awards and numerous publications and presentations. BioHybrid treated diabetic rodents with active microspheres and reversed their diabetes for up to 12 months. However, when the project ended in 1999, the treatment of larger animals, such as dogs and monkeys, still required immune suppression drugs to prevent rejection of the microspheres. Although BioHybrid considers microsphere technology viable, the company needed an additional $40 million for further development beyond the $38 million they invested prior to, during, and subsequent to the ATP award. BioHybrid was unable to raise the necessary funds. Furthermore, industry focus shifted away from implantable microsphere technology to stem cell research. The lack of funding coupled with the lack of interest in the industry forced BioHybrid to put the project on hold.

After the ATP-funded project ended, Synergy (renamed Synergy Innovations, Inc.) switched to developing micron-scale powders used in soldering metals. Development of these powders, called MonoSphere Powders, was partly based on the ATP-funded technology developed during this project. Synergy expects that these coatings will be used for manufacturing miniature circuits (such as for cell phones and global positioning systems) and for many other applications. Since 2000, the company has earned over $1 million in research/contract revenue due in part to this ATP-funded technology.

COMPOSITE PERFORMANCE SCORE

(based on a four star rating)

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Research and data for Status Report 93-01-0094 were collected during March – May 2005.

Disease Treatments Are Often Imprecise

Disease treatments have included injections, oral pharmaceuticals, and organ transplants; each treatment method, however, presents limitations and challenges. The first successful human organ transplant was performed in 1954: a kidney was transplanted from an identical twin donor. Since then, medical science has extended the basic technology to include the heart, pancreas, kidney, lung, and liver, with varying success. One major obstacle to wider use of cell and tissue transplants is the body's own immune system. The

procedure requires that patients take powerful immunosuppressive drugs, which can cause a variety of serious complications including cancer, infection, renal failure, and osteoporosis. Other concerns include a shortage of available human donor tissue and the medical risk of invasive surgery.

Alternative approaches to treating human and animal diseases rely on periodic injections or oral administrations of pharmacologically active substances. Dosages are somewhat imprecise, and diet changes frequently alter the body's requirements. Furthermore, a patient may miss or delay a dosage, thus compromising the treatment's effectiveness.

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A patient with Type 1 (insulin-dependent) diabetes, for example, is typically treated through frequent blood glucose monitoring and insulin injections or occasionally through a pancreas transplant. A promising alternative treatment method, cell therapy, was to implant in the patient specific healthy living pancreas cells that naturally produce insulin needed by the patient. The cells would be small enough that they could be injected with a hypodermic needle, eliminating the need for complex surgery.

Furthermore, cell therapy offered the potential to replace frequent injections and oral medications with much less frequent injections of living cells. A healthy pancreas provides natural, physiologic control of sugar. The normal function is provided by a small portion of the pancreas, which contains Islets of Langerhans, including insulin-producing beta cells.

BioHybrid Proposes Implanted Microspheres

In their 1993 ATP project proposal, BioHybrid Technologies and its joint venture partner, Synergy Research Corp., planned to encase transplanted living cells in microspheres (capsules). Synergy's primary role was to develop the encapsulation technology, which would prevent host rejection. The joint venture would collaborate with several laboratories for the animal studies: Tufts University, School of Veterinary Medicine; Worcester Foundation for Experimental Biology; Massachusetts Institute of Technology; Pine Acres Research Facility; and Mason Laboratories.

BioHybrid would first target the treatment of diabetes. The microsphere would be less than 600 micrometers (a micrometer is one-millionth of a meter) in diameter, could contain multiple living insulin-producing islets, and could be implanted in the patient by injection, avoiding difficult and risky surgery. Implanted microspheres containing islets could simulate insulin production provided by a normally functioning pancreas. When blood glucose levels rise, the change would be detected by implanted islets, which would release appropriate amounts of insulin into the body.

To avoid transplant rejection, the islets would be encased in semi-permeable microspheres to isolate them from the immune system. These microspheres would have additional coating(s) with pores large enough to permit glucose, nutrients, electrolytes, oxygen, and insulin to pass. However, the pores would be small enough to block other relatively bulky molecules involved in transplant rejection. Thus the coatings would prevent the body from rejecting the foreign islet cells contained within the microspheres, while keeping them alive and allowing them to function properly. This was the primary technical risk.

Isolating the implanted cells from the immune system by using microspheres opened the possibility of using cells from animal sources (for example, pancreatic islets from healthy pigs or calves for diabetes treatment), an option that would address the shortage of human organ transplants. BioHybrid also envisioned similar applications to treat other diseases in the future: hemophilia, Huntington's Chorea, Parkinson's disease, Alzheimer's disease, liver failure, AIDS, and cancer. These diseases require hormone, enzyme, or factor replacement (for example, one such factor is an essential part of the blood that is responsible for clotting).

Because of the high technical risk, BioHybrid was unable to obtain commercial funding. Therefore, ATP awarded cost-shared funding for three years of

technical development, beginning in 1994. (The project was later extended at no cost to ATP for two additional years.)

Microspheres Keep Cells Alive

The BioHybrid team needed to address three development areas: 1) bioprocess engineering; 2) microsphere design, production, and function; and 3) animal testing of microspheres containing living islet cells.

The bioprocess engineering requirements for this project included understanding the behavior of conducting liquids in strong electric fields, the effect of such fields on mammalian cells, and the mechanisms of microdroplet formation in such fields. The original design would undergo numerous adjustments in response to progress and results.

Microsphere development was a key support technology and had three parts:

- **Selecting the polymer**. BioHybrid and Synergy had to select the optimal polymer to be used as the shell to protect the islets. They decided to use alginate, because of its biocompatibility. It has long been used in medical applications, such as skin grafts, dental implants, and wound coverings.

- **Optimizing the composition of the outside coating**. The team had to experiment with the various characteristics of the microsphere coating(s), such as coating thickness, number of coats, and size of pores. These variables could cause chemical alterations in the alginate core.

- **Developing coating procedures**. The team used a film coater and tested it for application to the exterior surface of microspheres. They then altered flow rate, electrical field strengths, and electrode configuration in order to produce microspheres with a diameter of 400 to 1,000 micrometers.

This work led to in vitro analyses using culture media and, ultimately, to animal studies where they first treated small diabetic animals (mice) and later progressed to larger animals (rats, dogs, and monkeys).

Treatment of Diabetic Rodents Succeeds

BioHybrid successfully derived living bovine, porcine, and canine islet cells in increasingly larger quantities. Their yields from any given pancreas were high. The average number of islets per isolation increased from 514,000 at the start of the project to nearly 1 million (with several isolations over 2 million) by the end. They found that porcine sources of a particular species and size gave the best results. The harvested pancreas islets measured 50 to 200 micrometers.

BioHybrid Technologies and its joint venture partner, Synergy Research Corp., planned to encase transplanted living cells in microspheres (capsules).

BioHybrid encased pancreas islets in microspheres. They experimented with microsphere sizes ranging from 750 to greater than 2,000 micrometers that contained multiple islets. They eventually changed the microsphere material to a more purified form of alginate called Pronova UP-LVG. The company earned six patents for techniques related to microsphere production and use. They experimented with varying concentrations of islets (low densities of 25,000 to 50,000 islets per cm^3 and high densities of 75,000 to 125,000 islets per cm^3). Low densities were more effective, because the microspheres with high-density islets resulted in more fibrosis (fibrosis is the immune system's response by forming fibers around a foreign object, an early indicator of the transplant rejection process). This fibrotic response would restrict the flow of nutrients through the microsphere.

By 1996, BioHybrid's implantable microspheres successfully treated diabetic mice and rats for up to 12 months with no immune response or rejection. After they had perfected the process on smaller animals, the company started treating larger animals.

Funding for Large Animal Treatment Falls Short

In late 1996, BioHybrid requested a no-cost extension to the ATP-funded project, because progress was slower than anticipated. By the end of the project in 1999, diabetic dogs with transplanted islets in

microspheres were able to function for up to six months. However, immunosuppressive medications were required to prevent a fibrotic response. BioHybrid determined that more development was needed before large animals could be successfully treated.

Altogether, BioHybrid invested $38 million in outside funding before, during and after their ATP-funded project. In the mid- to late 1990s, the company entered into negotiations with several major pharmaceutical firms that were considering supporting continued development. These firms included GlaxoSmithKline, Merck, and Novartis. BioHybrid needed to provide 30 days of good data with large animals to demonstrate viability. The company was unable to do so, because the animals were still having problems with fibrotic response to the microspheres. Consequently, BioHybrid was unable to fund the necessary additional large-animal studies. After project conclusion, the company raised $2 million in additional research funding from the Juvenile Diabetes Research Foundation and private sources, but funding slowed dramatically in late 2001. BioHybrid estimated that it needed at least $40 to $50 million in additional support to bring the research to human clinical trials. Funding was short, and the biotechnology field shifted its interest to stem cell research as a potential source for cell therapy. Competitor firms have switched to stem cell research, but none has yet entered human clinical trials. Although its research is currently on hold, BioHybrid still believes implantable microsphere technology from animal sources is viable and hopes to develop animal-source microsphere therapeutics in the future.

BioHybrid's implantable microspheres successfully treated diabetic mice and rats for up to 12 months with no immune response or rejection.

As of 2005, BioHybrid's sister company, Sensor Technologies, was developing a sensor that diabetic patients can use to read their glucose levels in real time. An implant is placed under the patient's skin, and the sensor, about the size of a watch, is positioned over the implant. The implant uses a capsule based in part on the microsphere technology advanced during the ATP-funded project. This method eliminates the need to draw blood, a necessary step in existing glucose-monitoring techniques.

Synergy Develops Uniform, Spherical Powders

During their work on encapsulating cells for BioHybrid's pancreatic islets, Synergy (renamed Synergy Innovations, Inc.) enhanced its technical skills for fabricating uniform-sized, spherical powders and applied this to monosphere-powder manufacturing. The company was awarded more than $800,000 in National Science Foundation Small Business Innovation Research projects to continue development. Synergy used the technique developed during the ATP-funded project to make solder powder used in electronic solder paste, which they called MonoSphere Powder. The benefits from this project include:

- Cooling and solidifying droplets without deforming them (sizes range from 4 to 750 micrometers)
- Electrically charging droplets to prevent clustering during the cooling and solidification process
- Preventing oxidation of ultrafine solder powder through proprietary coatings

Synergy has applied for a patent on these advances. Commercial applications include higher component density on electronic printed circuit boards. Solder paste can be dispensed under computer control. This will facilitate miniaturizing circuits for electronic products, including cell phones, global positioning systems, miniature robots, and unmanned aerial vehicle aircraft. Since 2000, Synergy has sold over $1 million in research-contract services that were partially based on the ATP-funded microsphere technology. As of 2005, Synergy was in negotiations with a well-known powder manufacturer to license the electronic solder powder process. Sales are expected to grow in other markets as well. A spin-off company, MonoSphere Powders, Inc. expects to be incorporated in the future.

Conclusion

BioHybrid Technologies, Inc. and its joint venture partner, Synergy Research Corp. (later renamed

Synergy Innovations, Inc.), submitted a proposal in 1993 to develop implantable microspheres (semi-permeable “capsules”) for diabetic patients, which would contain healthy living pancreas cells from animal sources. The key to success would be to develop the microspheres and appropriate coatings to allow the cells to stay alive by permitting nutrients, waste, insulin, and similar molecules to pass, but to prevent the passage of the larger molecules associated with transplant rejection. The ATP-funded project ran from 1994 to 1999. BioHybrid was awarded six patents related to microsphere production, published numerous articles, and shared research findings through presentations. Although the companies developed useful microspheres and coatings and successfully treated diabetic mice and rats for up to 12 months, they were unable to avoid immune rejection in larger animals (dogs and monkeys). The studies have not reached human clinical trials. However, Synergy was able to use the microsphere technology to develop MonoSphere solder powders used in miniature electronic circuits.

PROJECT HIGHLIGHTS
BioHybrid Technologies, Inc.

Project Title: Treating Disease Using Implanted Living Cells in Microspheres (Disease Treatment Using Living Implantable Microreactors)

Project: To develop implantable microspheres containing living cells isolated from the body's immune system to treat diseases requiring replacement of hormones, enzymes, factors, and other cell-produced bioagents.

Duration: 4/1/1994 - 3/31/1999
ATP Number: 93-01-0094

Funding (in thousands):

ATP Final Cost	$4,260	49.8%
Participant Final Cost	4,288	50.2%
Total	$8,548	

Accomplishments: While BioHybrid Technologies, Inc. and Synergy Innovations, Inc. did not reach clinical trials for implantable microspheres, they did advance the use of microspheres and their coatings. They made the following technical advances:

- BioHybrid harvested porcine pancreas islet cells in larger quantities than competitors had achieved. Yields per isolation increased from 514,000 to almost 1 million. The harvested pancreas islets measured 50 to 200 micrometers.
- BioHybrid and Synergy developed microspheres measuring 750 to greater than 2,000 micrometers in diameter. They altered the microsphere material to a more purified form of alginate called Pronova UP-LVG and developed protective coatings that would allow small molecules to pass, but block the large molecules involved in transplant rejection. Low-density concentrations of islets (25,000 to 50,000 islets per cm^3) were more effective in preventing rejection.
- By 1996, BioHybrid's implantable microspheres successfully treated diabetic mice and rats for up to 12 months with no deleterious immune response or rejection. They had similar results with larger animals, but had to use medication to suppress immune response.
- As of 2005, Sensor Technologies, BioHybrid's sister company, was developing a sensor that diabetic patients would use to read their glucose levels in real time. An implant is inserted under the patient's skin, and the sensor, which is about the size of a watch, is placed over the implant. The implant uses a capsule based in part on the microsphere technology advanced during this ATP-funded project. This method will eliminate the blood draws necessary for existing glucose monitoring.

Although the original therapeutic application to treat diabetes stalled, Synergy applied its ATP-funded technology to other uses. The company improved its processes for producing micron-scale powders and coatings and has applied for a patent on these advances. Partially based on this project, they have learned to:

- Cool and solidify droplets while maintaining uniform shape (sizes ranged from 4 to 750 micrometers)
- Electrically charge droplets to keep them separated during the cooling
- Prevent oxidation of ultra-fine solder powder

BioHybrid earned the following six patents for microsphere production and use:

- "Methods of use of uncoated gel particles" (No. 5,651,980: filed April 15, 1994; granted July 29, 1997)
- "Microcapsules and composite microreactors for immunoisolation of cells" (No. 6,126,936: filed March 10, 1995; granted October 3, 2000)
- "Methods of use of uncoated gel particles" (No. 5,912,005: filed November 19, 1996; granted June 15, 1999)
- "Non-steroidal anti-inflammatory agents inhibition of fibrotic response to an implanted device" (No. 5,891,477: filed March 28, 1997; granted April 6, 1999)
- "Devices containing cells or tissue and an agent that inhibits damage by a host cell molecule" (No. 6,287,558: filed August 1, 1997; granted September 11, 2001)
- "Device for cloaking transplanted cells" (No. 6,368,612: filed December 24, 1997; granted April 9, 2002)

PROJECT HIGHLIGHTS
BioHybrid Technologies, Inc.

Commercialization Status: BioHybrid was unable to commercialize its microsphere technology for living islet cells because the cost of additional necessary large-animal testing was too high ($40–$50 million beyond the $38 million they invested before, during, and after the ATP award). However, Synergy has had success in using the ATP-funded technology in its MonoSphere Powder. Synergy's MonoSphere solder powder is unique and can be used in electronic solder paste in electronic solder paste. The paste can be dispensed under computer control to enhance the miniaturizing of circuits for electronic products, including cell phones, global positioning systems, miniature robots, and unmanned aerial vehicles. The paste-dispensing system has not entered the market yet.

Outlook: Because other technologies have changed research priorities, the outlook for implantable microspheres is poor. However, the microsphere technology developed in part during this project is being used to develop a bloodless glucose sensor for diabetic patients and micron-scale electronic solder powder. The MonoSphere Powders can be used for miniaturizing circuit boards in many electronic products.

Composite Performance Score: * *

Number of Employees: 16 employees at project start, 0 as of April 2005 (BioHybrid Technologies); 40 employees at project start, 42 as of March 1998, and 5 as of April 2005 (Synergy Innovations).

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Publications: BioHybrid researchers disseminated their findings through the following publications:

- Lanza, R. P., and W. L. Chick. "Encapsulated Cell Transplantation." *Transplantation Reviews*. Vol. 9, No. 4, pp. 1-15, 1995.
- Lanza, R. P., W. M. Kuhtreiber, D. M. Ecker, J. P. Marsh, J. E. Staruk, W. L. Chick. "Successful Bovine Islet Xenografts in Rodents and Dogs Using Injectable Microreactors." *Transplantation Proceedings*. Vol. 27, No. 6, p. 3322, 1995.
- Lanza, R. P., W. M. Kuehtreiber, D. Ecker, J. E. Staruk, and W. L. Chick. "Xenotransplantation of Porcine and Bovine Islets without Immunosuppression Using Uncoated Alginate Microreactors." *Transplantation*. Vol. 59, No. 10, pp. 1377-84, May 1995.
- Lanza, R. P., and W. L. Chick. "Encapsulated Cell Therapy Scientific." *American Science & Medicine*. Vol. 2, No. 4, p. 16, 1995.
- Lanza, R. P., D. M. Ecker, W. M. Kuhtreiber, J. E. Staruk, J. Marsh, and W. L. Chick. "A Simple Method for Transplanting Discordant Islets into Rats Using Alginate Gel Spheres." *Transplantation*. Vol. 59, pp. 1485-1487, 1995.
- Lanza, R. P, W. M. Kuhtreiber, J. P. Marsh, and W. L. Chick. "A Simple and Inexpensive Method for Transplanting Xenogeneic Cells and Tissues Into Rats Using Alginate Spheres." *Transplantation Proceedings*. Vol. 27, No. 6, p. 3322, December 1995.
- Lanza, R. P., W. M. Kuhtreiber, J. P. Marsh, and W. L. Chick. "Transplantation of Porcine and Bovine Islets Into Mice Without Immunosuppression Using Uncoated Alginate Microspheres." *Transplantation Proceedings*. Vol. 27, No. 6, p. 3321, December 1995.
- Lanza, R. P., J. L. Hayes, and W. L. Chick. "Encapsulated Cell Technology: Moving Into the Marketplace." *Nature Biotechnology*. Vol. 14, No. 9, p. 1107, 1996.
- Lanza, R. P., and W. L. Chick. "Commentary on Controlled-Release of Antibodies for Long-Term Topical Passive Immunoprotection of Female Mice Against Genital Herpes." "Slow Release Devices: STD Protection at Last." *Nature Biotechnology*. Vol. 14, No. 2, p. 437, 1996.

PROJECT HIGHLIGHTS
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- Lanza, R. P., W. M. Kuhtreiber, D. M. Ecker, J. P. Marsh, J. E. Staruk, and W. L. Chick. "Xenotransplantation of Bovine Islets Into Dogs Using Biodegradable Microreactors." *Transplantation Proceedings*. Vol. 28, No. 2, April 1996.

- Lanza, R. P., and W. L. Chick. "Transplantation of Encapsulated Cells and Tissues." *Surgery*. Vol. 121, No.1, January 1997.

- Lanza, R. P., and W. L. Chick. "Immunoisolation Strategies for the Transplantation of Pancreatic Islets." "Bioartificial Organs: Science, Medicine and Technology." *New York Academy of Sciences*. Vol. 831, pp. 323-331, 1997.

- Lanza, R. P., and W. L. Chick. "Immunoisolation at a Turning Point." *Immunology Today*. Vol. 18, No. 3, March 1997.

- Lanza, R. P., D. K. C. Cooper, and W. L. Chick. "Xenotransplantation." *Scientific American*. July 1997.

- Lanza, R. P., D. M. Ecker, W. M. Kuhtreiber, J. P. Marsh, J. Ringling, and W. L. Chick. "Transplantation of Islets Using Microencapsulation Studies in Diabetic Rodents and Dogs." *Journal of Molecular Medicine*. Vol. 77, No. 1, pp. 206-10, January 1999.

- Lanza, R. P., R. Jackson, A. Sullivan, C. McGrath, W. Kuhtreiber, and W. L. Chick. "Xenotransplantation of Cells Using Biodegradable Microcapsules." *Transplantation*. Vol. 67, No. 8, pp. 1105-11, April 1999.

Presentations:

- Lanza, R. P., W. M. Kuehtreiber, and W. L. Chick. "Islet Transplantation Using Immunoisolation." Congress of the International Society for Artificial Cells, Blood Substitutes, and Immobilization Biotechnology 22(5): A26, 1994. XI. Boston, MA, 1994.

- Ecker, D. M., R. P. Lanza, W. M. Kuehtreiber, J. E. Staruk, J. P. Marsh, and W. L. Chick. "Transplantation of Discordant Islet Xenografts Using Uncoated Alginate Microreactors." American Society for Artificial Internal Organs 41. Chicago, IL, 1995.